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COMMENTARY

Structure and dynamics of hepatic endothelial fenestrae

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TEXT

Liver sinusoids can be regarded as unique capillaries that differ from other capillaries by the presence of endothelial cells with open fenestrae lacking a diaphragm and a basal lamina. Fenestrae are dynamic structures that act as a sieve controlling the extensive exchange of material between the blood and the parenchymal cells. Alterations in the number or diameter of fenestrae by drugs, hormones, toxins, and diseases can produce serious perturbations in liver function.

Few studies have established the involvement of the cytoskeleton in the regulation of the number and size of fenestrae. Although a specialized cytoskeleton is clearly associated with fenestrae, evidence at the ultrastructural and biochemical level is limited. No mechanism or structure has been described to explain the changes in the number of fenestrae. Therefore, the aim of our study is to investigate the fine structure of fenestrae by using a variety of high-resolution microscopic methods, and to study the effect of drugs, known to interfere with the cytoskeleton, on the dynamics of fenestrae. In order to investigate these aspects under standardized conditions, it was necessary to develop a method for the isolation, purification and cultivation of liver sinusoidal endothelial cells (LSEC).

LSECs were isolated by collagenase perfusion of the liver, isopycnic sedimentation in a two-step Percoll gradient, and selective adherence to different substrates. LSECs isolated by this method provide a vital and responsive cell population enabling the study of structure and function of these cells *in vitro*. Detergent-extracted whole mounts of LSECs show an integrated cytoskeleton. Sieve plates and fenestrae are both delineated by a filamentous sieve plate, and fenestrae-associated cytoskeleton ring (FACR). Because of the fact that the FACR opens and closes like fenestrae in response to different treatments, it is assumed that this ring probably regulates the size changes of the fenestrae. Treatment of LSECs with various microfilament disrupting agents shows that the actin cytoskeleton determines the number of fenestrae. Using microtubule-altering agents we could demonstrate that microtubules are involved in the increase of the number of fenestrae after microfilament disruption. Furthermore, by treating LSECs with the actin inhibitor misakinolide, we were able to capture a structure indicative of fenestrae formation, which we propose to call fenestrae forming center (FFC). Recently we have used new actin binding agents discovered by the Stony Brook Group that belong to a large family of structurally related marine macrolides isolated from various pacific sponges. The mechanism of action of these novel actin-perturbing drugs is still under study, but they appear to exhibit barbed-end capping and F-actin severing activity. Interestingly, besides misakinolide, we found that one of these new agents is also able to reveal the process of fenestrae formation. Therefore, the unmasking of nascent fenestrae, emerging from the FFCs only by two different agents, indicates once more that specific alterations in actin organization at particular locations, and at particular times are required to bring to light the process of fenestrae formation. In addition, in order to study the process of fenestrae formation in living LSECs we applied atomic force microscopy. Unfortunately, we demonstrated that the resolution of the stomic force microscope using living LSECs was poor as compared to dried-coated, dried-uncoated and wet-fixed cells. This difference in resolution could be explained by the difference in elasticity between living and fixed LSECs.

In conclusion, we demonstrated that fenestrae are delineated by a filamentous fenestrae-associated cytoskeleton which plays a role in maintaining and regulating the size of fenestrae after different treatments. In addition, our findings unambiguously show the involvement of special domains in *de novo* formation of fenestrae and focuses future research on the molecular composition of the FFC and the FACR.

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